Citation:

Kilonzo-Nthenge A, Chen FC, Godwin SL. Efficacy of home washing methods in controlling surface microbial contamination on fresh produce. J Food Prot. 2006 Feb; 69(2): 330-334.

PubMed ID: 16496573

Study Design:

Non-randomized trial.

Class:

C - Click here for explanation of classification scheme.

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To investigate the effectiveness of different cleaning methods that can be used by consumers in reducing microbial contamination on fresh produce (lettuce, broccoli, apples and tomatoes) in a home setting.

Inclusion Criteria:

Samples of lettuce, tomatoes, apples and broccoli purchased from local grocery store in Nashville, Tennesee.

Exclusion Criteria:

Samples of lettuce, tomatoes, apples and broccoli that were bruised or had cracks.

Description of Study Protocol:

Recruitment

Samples of lettuce, tomatoes, apples, and broccoli were purchased from local grocery store in Nashville, Tennessee, on the day before the experiment and stored in their original boxes at 4°C.

Design

Non-randomized trial.

Intervention

- Bacterial strain and inoculation preparation:
  - Bacterial strain used was Listeria innocua (ATCC, 33090) which was used as

a surrogate for L. monocytogenes
    - Inoculum was applied to the selected fresh produce within one hour of preparation
    - Concentration of inoculum was determined by surface plating of serially diluted culture into Listeria selective agar with supplement and plates were incubated at 37◦ for 48 hours before the colonies were counted
- Procedure for inoculating fresh produce:
    - Lettuce, tomatoes, apples and broccoli were used as models to represent fresh produce of different surface characteristics
    - Samples were purchased from a local grocery store on the day before experiment and stored in original boxes at 4◦C
    - Apples were pre-treated in warm water before inoculation to remove wax coating that might interfere with bacteria attachment to the fruit
    - Fresh produce was individually submerged in three liters of bacterial inoculum (about 108 CFU per ml) and agitated by stirring with a sterilized stainless steel spoon for three minutes
    - Inoculated produce was air dried for 10 minutes in a biosafety cabinet before subjected to treatments
    - Positive controls for each produce were analyzed to determine baseline level of L. innocua
- Produce cleaning procedure: Inoculated samples were subjected to combinations of cleaning procedures
    - First:
        - Soaked for two minutes in tap water (room temperature or warm at 40◦C)
        - Veggie Wash solution (2.0oz per gallon of water)
        - 5% vinegar solution
        - 13% lemon solution
    - Second:
        - Rinse with cold tap water (15 seconds), or
        - Wipe with wet or dry paper towel (15 seconds)
    - During soaking, produce was rotated to ensure full surface coverage
    - Efficacy of soaking in tap water followed by rinse step was also tested for stem and blossom end of apple and for flower and stem sections of broccoli
    - Each kind of produce had three replicates for treatment per experiment, and each experiment was performed twice
- Microbiological analysis:
    - Populations of L. innocua on treated samples were determined by plating 0.1ml of serially diluted homogenized sample on Listeria selective agar
    - Three replicates of each sample were analyzed and each replicate included a minimum of three plates with serial dilutions.

Statistical Analysis

- Microbiological counts (CFU per gram) of samples and the data were transformed to log reduction before statistical analysis
- Log reductions for each treatment were compared for statistical significance by the GLM procedure with SPSS-PC
- Differences of means for treatments were separated by least significant difference at $P<0.05$.

Data Collection Summary:

Timing of Measurements

- Three replicates of each sample were analyzed per experiment and each replicate included a minimum of three plates with serial dilutions; each experiment was preformed two times
- Plates were incubated for 48 hours at 37◦C before the colonies were counted.

Dependent Variables

Listeria innocua (ATCC, 33090) (used as a surrogate for L. monocytogenes).

Independent Variables

- Cleaning procedures and materials used:
  - Soak for two minutes in:
    - Tap water (room temperature or warm at 40◦C)
    - Veggie Wash Solution (2.0oz per gallon of water); Beaumont Products, Inc.
    - 5% vinegar solution
    - 13% lemon solution
  - Rinse with:
    - Cold tap water (15 seconds)
    - Brush under running tap water (15 seconds)
    - Wipe with wet or dry paper towel (15 seconds)
- Type of produce (lettuce, broccoli, apples, tomato)
- Parts of fruits or vegetables (stem and blossom of apples, flower and stem of broccoli) inoculated
- Recovery method (stomacher for lettuce and broccoli; bacteria detached from surface by hand rubbing for two minutes in peptone water for apple and tomatoes).

Control Variables

Procedure for inoculating fresh produce (except for apples that were pre-treated in warm water for two minutes to remove wax coating) in terms of procedures for inoculation.

Description of Actual Data Sample:

- Initial N: Six samples each of lettuce, broccoli, apples and tomatoes
- Location: Tennessee.

Summary of Results:

General Findings

- Pre-soaking in water before rinsing significantly reduced bacteria in apples, tomatoes and lettuce, but not in broccoli

- Wiping apples and tomatoes with wet or dry paper towel showed lower bacterial reductions compared with soaking and rinsing procedures
- Blossom ends of apples were more contaminated than the surface after soaking and rinsing; similar results were observed between flower section and stem of broccoli
- Reductions of L. innocua in both tomatoes and apples (2.01 to 2.89 log CFU per g) were more than in lettuce and broccoli (1.41 to 1.88 log CFU per g) when subjected to same washing procedures
- Reductions of surface contamination of lettuce after soaking in lemon or vinegar solutions were not significantly different (P>0.05) from lettuce soaking in cold tap water.

Specific Findings Related to Types of Produce

- Lettuce:
  - Rinsing lettuce leaves under cold running tap water for 15 seconds without prior soaking showed the lowest significant (P<0.05) bacterial reduction of 1.41 log CFU per g among all other treatments
  - Reductions of L. innocua populations ranged from 1.72 to 1.88 log CFU per g after soaking lettuce leaves in 13% lemon, 5% vinegar, Veggie Wash solution or tap water (room temperature or warm at 40◦C) for two minutes, followed by a 15-second rinse under running tap water
  - Soaking lettuce leaves in lemon and vinegar solutions showed no difference (P>0.05) in bacterial reductions from soaking in cold tap water for two minutes
- Broccoli:
  - Similar to the observations in lettuce, soaking broccoli in either cold water, warm water or Veggie Wash solution before rinsing improved the reduction compared with rinsing only (1.41 log CFU per g), although the differences were not significant
  - Based on separate analyses, a higher contamination level was found on the flower (7.47 log CFU per g) than on the stem portion (6.93 log CFU per g); also, the flower showed greater reduction (1.49 log CFU per g) than the stem portion (0.46 log CFU per g) after soaking in water for two minutes followed by a rinse step
- Apples:
  - Soaking before rinsing (2.32 log CFU per g) significantly improved bacteria reduction compared with rinsing alone (2.01 log CFU per g)
  - Wiping apples with dry or wet paper towel had the lowest significant (P<0.05) reductions of 0.66 and 0.96 log CFU per g, respectively, among all treatments
  - Stem and blossom ends had higher residual contamination of L. innocua (3.70 log CFU per g) than the surface (1.18 log CFU per g) after soaking in water followed by rinsing
- Tomatoes:
  - Veggie Wash solution had a reduction of 2.89 log CFU per g and was significantly higher (P<0.05) than all other cleaning methods
  - Rinsing (15 seconds) under running tap water without prior soaking had a significantly lower bacterial reduction of 2.10 log CFU per g than rinsing with rubbing without pre-soaking (2.36 log CFU per g) or rinsing after soaking (2.53 log CFU per g)
  - Wiping with a wet or dry paper towel without soaking or rinsing had

significantly low bacterial reductions of 1.94 and 1.85 log CFU per g, respectively. Generally, in both trials, bacterial reductions in each cleaning procedure was not significantly different (P>0.05), except in rinsing under cold tap water.

Author Conclusion:

- Results from this study suggest that washing produce under cold running tap water with rubbing and brushing, where applicable, has a potential to reduce surface bacterial contamination
- Education and extension personnel might consider it appropriate to instruct consumers to rub or brush fresh fruits and vegetables under cold running tap water before consumption.

Reviewer Comments:

- The inoculation level used in the experiment was higher than natural contamination to allow valid observation of bacterial reductions after different cleaning methods
- Small number of samples tested.

Limitations per Authors

- The model system used was designed to evaluate the effectiveness of cleaning methods after a short period of surface contamination on fresh produce, but in situations in which there is extended cold storage, Listeria can enter physiological states or associate with native microflora that make them more resistant to removal from produce surfaces.
- The nature of the different fruit and vegetable surfaces and the coating materials applied during processing might have affected the degree of attachment of bacteria, and how easily the bacteria were washed off when subjected to cleaning procedures.

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes

3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	???
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	N/A
2.4.	Were the subjects/patients a representative sample of the relevant population?	???
3.	**Were study groups comparable?**	N/A
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	N/A
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	N/A
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	N/A
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A

3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	N/A
4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	N/A
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	N/A
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	???
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	???
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes

6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	N/A
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	N/A
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	N/A
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	???
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	???
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	No

8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes